

The University of Chicago

RESPIRATION AND METABOLISM IN
ETIOLATED WHEAT SEEDLINGS
AS MODIFIED BY PHOSPHORUS
NUTRITION

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

1933

By
WINSTON W. JONES

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RESPIRATION AND METABOLISM IN ETIOLATED WHEAT SEEDLINGS AS INFLUENCED BY PHOSPHORUS NUTRITION

WINSTON W. JONES

(WITH TEN FIGURES)

Introduction

Phosphorus plays an important rôle in the process of respiration (12, 13, 29). It forms, in the presence of sugars, mono- and di-hexosephosphoric acid esters (8, 27), which are then broken down in the respiratory process. Under certain conditions it may form with sucrose a sucrose phosphoric acid ester (19). It is also known that plants starved for phosphorus differ considerably in their chemical composition from those not thus starved. MACGILLIVRAY (15) has pointed out that tomato plants starved for phosphorus show a decrease in coagulable nitrogen, and an increase in total nitrogen. There is also a marked increase in percentage of sugar. KRAYBILL (10) also showed that when phosphate is limiting in tomato plants nitrates as well as carbohydrates accumulate. According to ECKERSON (5) the lack of phosphorus causes a breakdown of the reductase activity of the plant, which is then followed by an accumulation of nitrates, sugars, and starch, and results in an essentially nitrogen-starved plant. It is desirable then to know the relation between respiration and the chemical composition of plants deficient in phosphorus as compared with plants not deficient in phosphorus.

In view of these facts and because phosphorus very often becomes limiting in agricultural soils the present investigation was undertaken. All of the chemical data cited above were obtained with green plants and after phosphorus starvation had become rather severe. It was the desire of the writer to determine some of the early symptoms of phosphorus starvation as shown by internal composition, as well as to measure the respiration during this period, and to relate any differences in composition to respiration, if possible. For these purposes large green plants are not suited; hence, etiolated seedlings with a considerable reserve of carbohydrates were used. Wheat was chosen as the most uniform plant at hand. Since LYON had obtained an increase in respiration owing to increased phosphate supply, the respiration behavior of wheat was reinvestigated, and, at the same time, the chemical changes caused by differences in phosphorus nutrition during early development were determined. It will be noted that the differences obtained in this work with different phosphorus treatments are small, as would be expected, since the seeds contain some phosphorus. The results, however, are in most cases consistent.

Materials and culture methods

CHOICE OF SEED

The seed used for this work had to meet two conditions: first, it must contain a relatively large stored food supply; and second, it must be small enough that a representative sample would not be unduly bulky. Wheat seemed to meet these requirements very well. A third desideratum was a low phosphorus content, but, as this quality depends somewhat on the nature of the soil in which the wheat is grown, a crop was grown to maturity in the greenhouse in a phosphorus-deficient nutrient solution. This crop yielded the seeds for the first part of the experiment, but owing to the small quantity of seeds obtained, field-grown seeds were used for the chemical studies. These field-grown seeds show respiratory responses to phosphate additions similar to those shown by the greenhouse-grown seeds, although the differences are not so marked. The seeds used were *Triticum vulgare* Vill. vars. Marquis and Poso, and *T. durum* Desf. var. Kubanka.

GREENHOUSE PLANTS

Marquis wheat was grown in the greenhouse in 2-gallon stone crocks filled with quartz sand. One set received each second day a nutrient solution containing phosphorus and the other set a solution lacking phosphorus. Distilled water was added when needed, and the pots were flushed once each week with distilled water. At the end of 23 days the plants without phosphorus were making such little growth that one application of the nutrient solution containing phosphorus was made. When the plants were mature the seeds were harvested and used in the respiration studies. They were placed in the respirometer chambers, with or without phosphorus, as described later under chemical methods. The nutrient solution used in each case throughout this work was made from 0.5 M stock solution the composition of which was as follows:

+ P		- P	
Salt	Concentration	Salt	Concentration
KH_2PO_4	0.005 M	KCl	0.005 M
$\text{Ca}(\text{NO}_3)_2$	0.005 M	$\text{Ca}(\text{NO}_3)_2$	0.005 M
MgSO_4	0.005 M	MgSO_4	0.005 M

In each case the total concentration of the nutrient solution was 0.015 M. It was adjusted to a pH of about 5.4.

FIELD SEED

Wheat seeds of the variety Poso were obtained from the United States Department of Agriculture Experiment Station at Davis, California, and the Kubanka from the North Dakota Agricultural Experiment Station. The

Marquis spring wheat was in the laboratory. In table I are shown the protein, starch, and phosphorus content of the seeds of these three varieties.

TABLE I
COMPOSITION OF FIELD-GROWN SEEDS IN PERCENTAGE OF DRY WEIGHT

WHEAT	YEAR OF CROP	WT. OF 100 SEEDS	PROTEIN	STARCH	PHOSPHORUS
		<i>gm.</i>	<i>%</i>	<i>%</i>	<i>%</i>
Marquis	1933	2.34	17.42	39.87	0.202
Poso	1935	2.53	11.35	42.10	0.050
Kubanka	1933	3.09	18.39	41.68	0.183

For chemical analysis, 25 gm. of seeds were sterilized in 0.25 per cent. uspulun for 20 minutes. Two sets of seeds were then germinated on cellulocotton. One set was moistened with a nutrient solution with phosphorus, the other set with a nutrient solution without phosphorus. After 24 hours' germination they were transferred to wire netting supported over a 4-gallon stone crock containing the nutrient solution. The nutrient solution was kept constantly stirred by means of a flowing air stream. They were grown for 7 days in a dark room at 27° C. A complete change of the nutrient solution was made each 48 hours. At the end of the 7-day period the plants were harvested and treated as described under chemical methods.

Chemical methods

RESPIRATION

DETERMINATION OF CARBON DIOXIDE.—The conductivity method was used for the determination of carbon dioxide. The absorption towers were similar in construction to those described by THOMAS (30). The towers were standardized and used as described by MITCHELL (17). The LiOH was 0.2 N strength. The respiratory flasks were 125-ml. Erlenmeyer flasks blackened on the outside to exclude light. The flasks containing the respiring seeds were placed, along with the absorption towers, in a constant temperature bath. The variation in temperature was $\pm 0.001^\circ$ C. Each respiration experiment was run in duplicate for 7 days. The procedure was as follows: One hundred seeds were sterilized in 0.25 per cent. uspulun for 20 minutes. The uspulun was then washed off with distilled water and the seeds were placed on moist cellulocotton in the respiratory flasks. The cellulocotton was moistened with the nutrient solution, either with or without phosphorus. The flasks were then placed in the water bath and the CO₂-free air stream turned on. One hour was allowed for the temperature adjustment. At the

end of this 1-hour period the air stream was turned through the absorption towers and the CO_2 absorbed for a 5-hour period, when the air was turned out of the absorption towers and the absorbed CO_2 determined. At the beginning of the second 24-hour period the CO_2 was again absorbed for a 5-hour period, so that at the end of 7 days the CO_2 had been determined for 7 five-hour periods.

FRACTIONATION AND EXTRACTION

At the end of the 7-day period the plants grown in the dark room were harvested and separated into tops, roots, and seed-remains. The samples taken were covered with hot 95 per cent. alcohol, enough being used so that the final concentration was 80 per cent. The samples were extracted as soon as possible with hot 80 per cent. alcohol, the alcohol being boiled each time on the steam bath for 15 minutes. The extract was allowed to cool to room temperature before filtering. Seven extractions were made.

CARBOHYDRATE FRACTIONS.—For sugars, an aliquot of the alcoholic extract was evaporated down and water added, and the water solution cleared with neutral lead acetate and potassium oxalate, as given by the Committee on Methods (2). The reducing sugar was then determined on this cleared solution by the bicarbonate modification of the Tompsett method as adapted and described by PHILLIPS (24). The residue was dried and ground so as to pass through an 80-mesh sieve and again dried. Starch was determined on this residue as described by NIEMANN *et al.* (20), and the reducing power of the resulting sugar determined as above for reducing sugar. No sucrose was found and starch was present only in the seed-remains.

NITROGEN FRACTIONS.—Total soluble nitrogen in the extract was determined by the reduced iron method of PUCHER, LEAVENWORTH, and VICKERY (26) modified and adapted to the micro-Kjeldahl method described by PREGL (25). One-ml. aliquots of the alcoholic extract were placed in the micro-Kjeldahl digestion flasks and 0.29 ml. of 1:1 H_2SO_4 and 0.086 gm. reduced iron added to each. The flasks were then shaken for 10 minutes and boiled over a low flame for 5 minutes. The contents were cooled and 0.86 ml. of concentrated H_2SO_4 and a knife point of a mixture of K_2SO_4 and CuSO_4 (1:3) were added to each. The digestion and distillation was then completed as described by PREGL. PREGL's method was also used for the determination of the insoluble nitrogen of the residue.

PHOSPHORUS FRACTIONS.—Soluble phosphorus was determined on 10-ml. aliquots of the alcoholic extract. The aliquot was evaporated almost to dryness, then digested, and phosphorus determined by the method described by COCKEFAIR (1). The phosphorus in the residue was determined on 100-mg. samples by the same method.

Data

RESPIRATION OF GREENHOUSE-GROWN SEEDS

The data for this paper will be presented in two parts: first, those dealing with the respiration of the greenhouse-grown seeds; and second, those dealing with the respiration and chemical composition of the field-grown seeds. Table II shows the composition of the greenhouse-grown seeds.

TABLE II

EFFECT OF PHOSPHORUS DEFICIENCY ON WEIGHT AND CHEMICAL COMPOSITION
OF MARQUIS WHEAT SEEDS

NUTRIENT	No. OF SEEDS FROM 22 POTS	WT. OF 100 SEEDS	PROTEIN	STARCH	PHOSPHORUS
			DRY WT. BASIS	DRY WT. BASIS	DRY WT. BASIS
		<i>gm.</i>	<i>%</i>	<i>%</i>	<i>%</i>
Deficient in P	1300	1.4646	15.80	37.40	0.444
Complete nutrient ...	1563	1.7101	15.02	44.60	0.688

Table II shows that the phosphorus-deficient plants produced fewer and smaller seeds, and that these seeds were slightly higher in protein but lower in starch and phosphorus than those seeds from the plants grown in a complete nutrient solution. Although the seeds from the phosphorus-deficient plants were much lower in phosphorus, yet they contained considerable of this element, this phosphorus having come from the original seed and from the one application of the phosphate-containing nutrient solution. In contrast with the data given in table II, GERICKE (6) obtained much more growth, earlier maturity, and greater fresh weight with wheat when grown

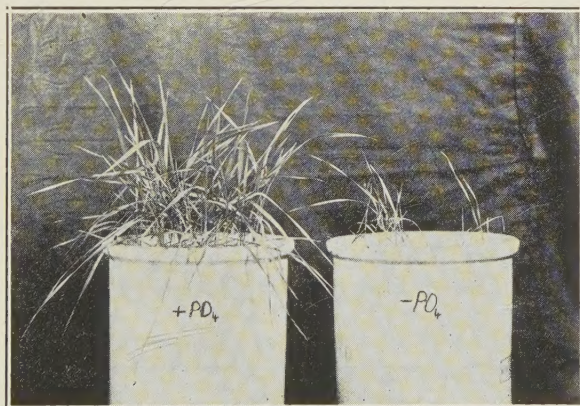


FIG. 1. Marquis wheat 28 days old grown in cultures with and without phosphorus.

in a nutrient solution lacking phosphate after the first 4 weeks of growth with phosphate.

Figure 1 shows the condition of Marquis spring wheat at 28 days of age in sand culture with and without phosphorus. These -P plants did not

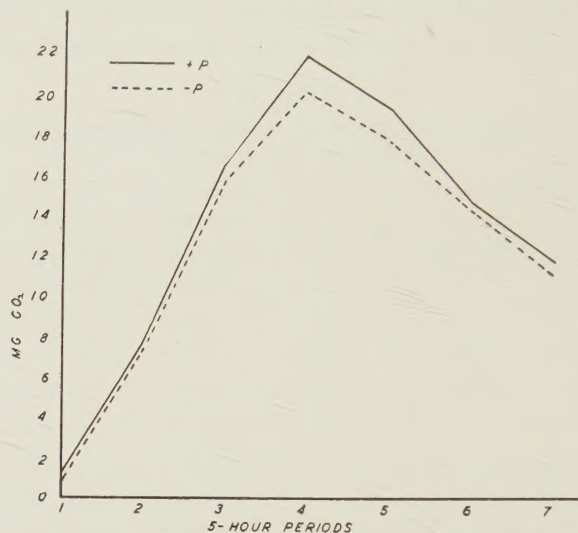


FIG. 2. Respiration of Marquis spring wheat seedlings. Seeds from plants not deficient in phosphorus. Data for graphs are in milligrams of CO_2 /5-hour period/gm. of air-dry seeds.

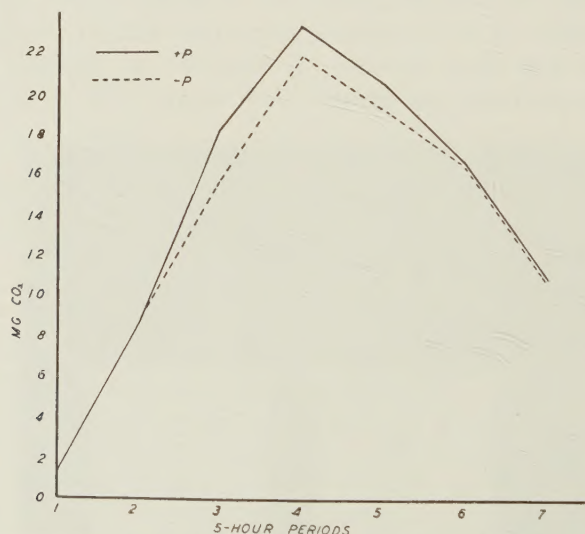


FIG. 3. Respiration of Marquis spring wheat seedlings. Seeds from plants deficient in phosphorus. Data for graphs are in milligrams of CO_2 /5-hour period/gm. of air-dry seeds.

receive phosphorus on the twenty-third day as did the ones from which the seeds were obtained. The plants shown died, in the case of the -P plants, a few days after the photograph was taken.

Table II gives the CO_2 produced per gram of air-dry seeds for the first 7 days after germination. The figures represent the amount in milligrams of CO_2 produced per gram per 5-hour period. The data for the seeds from deficient and non-deficient phosphorus plants when grown in solutions with and without phosphorus are given. This is represented graphically in figures 2 and 3.

TABLE III

CO_2 /5-HOUR PERIOD/GM. OF AIR-DRY SEEDS ON EACH OF THE FIRST 7 DAYS AFTER GERMINATION FOR MARQUIS WHEAT SEEDS DEFICIENT AND NOT DEFICIENT IN PHOSPHORUS

TREATMENT	MG. CO_2 /5-HOURS/GM.						
	1	2	3	4	5	6	7
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
+ P seeds in - P nutrient	0.58	7.41	15.71	20.05	17.64	14.23	11.11
+ P seeds in + P nutrient	1.10	7.54	16.42	21.80	19.21	14.70	11.80
- P seeds in - P nutrient	1.16	8.46	15.75	21.86	19.33	16.46	11.06
- P seeds in + P nutrient	1.16	8.46	18.20	23.33	20.60	16.66	11.09

When phosphorus is lacking in the nutrient solution of Marquis wheat seedlings, growth is greatly retarded, as shown in figure 1. If phosphorus is entirely lacking in the nutrient solution the wheat seedlings die after about 5 weeks. The phosphorus contained in the old seeds is not sufficient to carry the plants to maturity. When a small amount of phosphorus is supplied, so that phosphorus is still limiting but not lacking, the plants grow to maturity, but the total yield and weight per seed is much less for the phosphorus-deficient plants than for the plants not deficient in phosphorus. Furthermore, the chemical composition of the seeds from the phosphorus-deficient plants is different from that of the seeds from plants not deficient in phosphorus as shown in table II.

From table III and figures 2 and 3 it is shown that when phosphorus is deficient the amount of CO_2 evolved in respiration is reduced.

RESPIRATION OF FIELD-GROWN SEEDS

Table I shows the main constituents of the field-grown seeds. It will be noted that the Marquis spring wheat and Kubanka are both high in protein, and the Poso is low in protein. However, the protein content may vary

widely in a given variety as shown by MCGINNIS (18) and in table II, depending on the conditions under which the seeds were produced. It is interesting to compare the responses of the various wheats to phosphate. Table IV shows the CO_2 produced by each of the three wheats for a period of 7 days when grown in a complete nutrient solution and also when grown in a solution lacking phosphorus. The figures represent CO_2 /5-hour period/gm. as before. These data are shown graphically in figures 4, 5, and 6.

TABLE IV
 CO_2 /5-HOUR PERIOD/GM. OF AIR-DRY SEEDS ON EACH OF 7 DAYS FOR MARQUIS,
POSO, AND KUBANKA WHEAT SEEDLINGS

WHEAT	TREATMENT	MG. CO_2 /5-HOUR PERIOD/GM.						
		1	2	3	4	5	6	7
		mg.	mg.	mg.	mg.	mg.	mg.	mg.
Marquis	+ P nutrient	0.96	6.39	14.45	20.06	21.00	18.85	17.56
	- P nutrient	0.96	5.98	13.85	18.65	19.79	17.58	15.30
Poso	+ P nutrient	0.73	2.65	7.68	15.01	18.78	17.72	16.95
	- P nutrient	0.69	2.37	6.09	12.42	17.20	16.91	15.95
Kubanka ..	+ P nutrient	1.38	4.95	8.84	14.50	16.76	15.69	14.35
	- P nutrient	0.55	4.40	8.58	13.86	16.11	14.99	13.04

It is to be noted that, except for the first 5-hour period of the Marquis wheat, the CO_2 production of the plants growing in a complete nutrient solu-

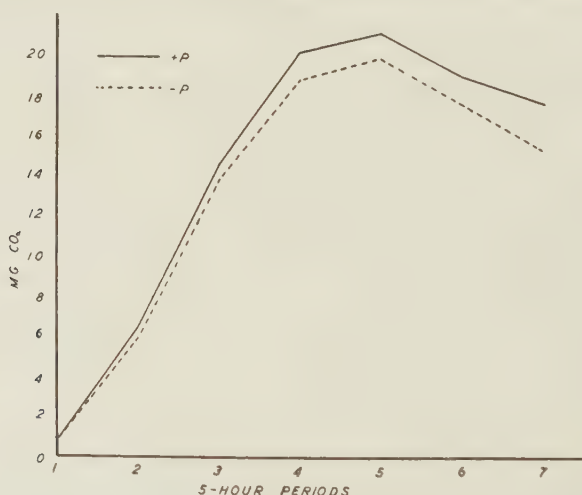


FIG. 4. Respiration of Marquis spring wheat seedlings, with and without phosphorus. Seeds from field-grown plants. Data for graphs are in milligrams of CO_2 /5-hour period/gm. of air-dry seeds.

tion is always greater than that of the plants in a solution lacking phosphorus. The respiration of both sets of plants increases to a maximum at the fifth 5-hour period, after which there is a slight decrease. At the end of the 7-day period other plants grown similarly were harvested and separated into tops, roots, and seed-remains. These were then analysed for reducing sugars, sucrose (none was found), starch, soluble nitrogen, insoluble nitrogen, soluble phosphorus, and insoluble phosphorus. These data are given in table V.

TABLE V

CHEMICAL COMPOSITION OF MARQUIS, POSO, AND KUBANKA WHEATS AFTER 7 DAYS GERMINATION IN THE DARK WITH AND WITHOUT PHOSPHORUS.

RESULTS IN PERCENTAGE OF DRY WEIGHT

VARIETY OF WHEAT	FRACTION	+ P			- P		
		TOPS	ROOTS	SEED	TOPS	ROOTS	SEED
		%	%	%	%	%	%
Marquis	Percentage of H ₂ O	91.41	93.49	81.08	90.69	93.27	80.35
	Reducing sugars	6.43	8.11	7.14	8.28	6.40	9.66
	Starch	None	None	15.01	None	None	12.68
	Soluble N	3.04	2.41	0.50	3.32	3.56	0.64
	Insoluble N	6.22	4.54	2.21	5.87	3.55	2.14
	Total N	9.26	6.95	2.71	9.19	7.11	2.78
	Soluble P	0.59	0.83	0.054	0.039	Trace	0.046
	Insoluble P	0.446	0.347	0.220	0.308	0.160	0.068
	Total P	1.036	1.177	0.274	0.347	0.160	0.114
Poso	Percentage of H ₂ O	91.85	93.76	79.78	91.92	93.62	78.09
	Reducing sugars	7.64	5.93	8.15	10.52	7.67	9.31
	Starch	None	None	13.75	None	None	16.70
	Soluble N	3.13	2.98	1.14	2.49	3.95	1.30
	Insoluble N	6.08	4.39	2.13	5.80	3.90	2.17
	Total N	9.21	7.37	3.27	8.29	7.85	3.47
	Soluble P	Trace	0.01	Trace	Trace	Trace	Trace
	Insoluble P	0.59	0.75	0.60	0.36	0.42	0.58
	Total P	0.59	0.76	0.60	0.36	0.42	0.58
Kubanka	Percentage of H ₂ O	91.68	92.56	85.25	91.09	92.72	76.70
	Reducing sugars	4.77	5.66	11.21	5.37	5.39	8.14
	Starch	None	None	14.95	None	None	18.74
	Soluble N	3.04	1.75	0.66	3.22	2.89	0.82
	Insoluble N	5.84	4.03	2.35	5.56	3.34	2.27
	Total N	8.88	5.78	3.01	8.78	6.23	3.09
	Soluble P	0.24	1.63	0.07	0.04	Trace	Trace
	Insoluble P	0.75	0.95	0.52	0.53	0.40	0.48
	Total P	0.99	2.58	0.59	0.57	0.40	0.48

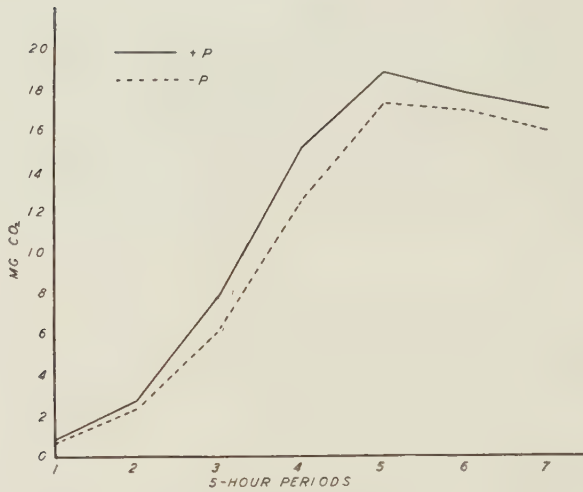


FIG. 5. Respiration of Poso wheat seedlings, with and without phosphorus. Seeds from field-grown plants. Data for graphs are in milligrams of CO_2 /5-hour period/gm. of air-dry seeds.

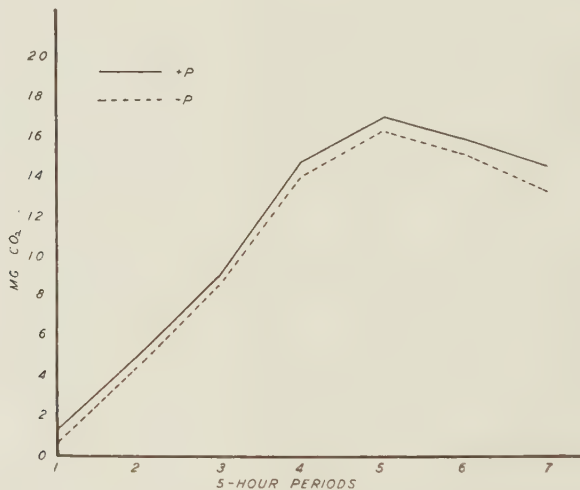


FIG. 6. Respiration of Durum (Kubanka) wheat seedlings, with and without phosphorus. Seeds from field-grown plants. Data for graphs are in milligrams of CO_2 /5-hour period/gm. of air-dry seeds.

Table V and figures 7, 8, 9, and 10 show that there is a very marked difference between plants grown on a minus-phosphorus nutrient solution and on a complete nutrient solution. First, the plants grown on the minus-phosphorus nutrient solution have a lower insoluble nitrogen content than those grown with phosphorus. Secondly, the -P plants have, in most cases, a

higher soluble nitrogen content than the + P plants. Furthermore, the reducing sugar content is, in most cases, higher in the - P plants, and yet the respiration of the - P plants is less than the + P plants (figs. 4, 5, 6). The difference in phosphorus content, especially soluble phosphorus, is very marked, as would be expected. In the plants lacking phosphorus in the nutrient solution there was only a trace of soluble phosphorus, while in the + P plants the soluble phosphorus was as high as 1.63 per cent. of the dry weight in one case.

Discussion

In the metabolism of plants phosphorus has several important functions (1). WOODWARD (32), as long ago as 1699, recognized the importance of phosphorus in the formation of plant bodies. Phosphorus occurs in the plant both in an inorganic form and in an organic form. VON OHLEN (31) shows that in germination there is a continual change of the organic phosphorus of the seed to the inorganic form which moves to the growing points where it again changes to the organic form. It may occur organically bound as nucleic acid (14), lecithin, cephalin, sphingomyelin (11), hexosediphosphate (8), hexosemonophosphate (27), and sucrosephosphate (19).

The present study concerns itself with the relation of phosphorus to protein and carbohydrate metabolism, and its relation to respiration.

RESPIRATION

That phosphorus is important in the process of respiration is shown by the work of HARDEN and YOUNG (8) on the fermentation of glucose by yeast-juice. These workers obtained large increases in the rate of fermentation and in the total amount of fermentation when they added a phosphate salt to the fermenting mixture of glucose and yeast-juice. The phosphorus forms with the glucose a hexosediphosphate which is then split up yielding an easily oxidized sugar and liberating the phosphorus. The phosphorus is then free to unite with more glucose. GOTTSCHALK (7), studying the action of a co-enzyme in connection with hexose phosphate, concludes that fermentation, caused by yeast, results in splitting off from glycogen small fractions of alpha, beta, or balanced glucose which is phosphorylated, perhaps by the aid of co-enzyme. From this phosphorylated compound is liberated a readily decomposable form of sugar which is susceptible to seizure by destructive enzymes. Such enzymes occur in plants (16) and could easily oxidize, or dehydrogenate, such unstable compounds as the hexose liberated from the hexosediphosphate. The rôle of phosphorus in respiration seems to be to form unstable substrates for the respiratory enzymes which attack these substrates and by their action liberate energy. For this purpose only small quantities of phosphorus are needed as it is not permanently bound. Other compounds, as

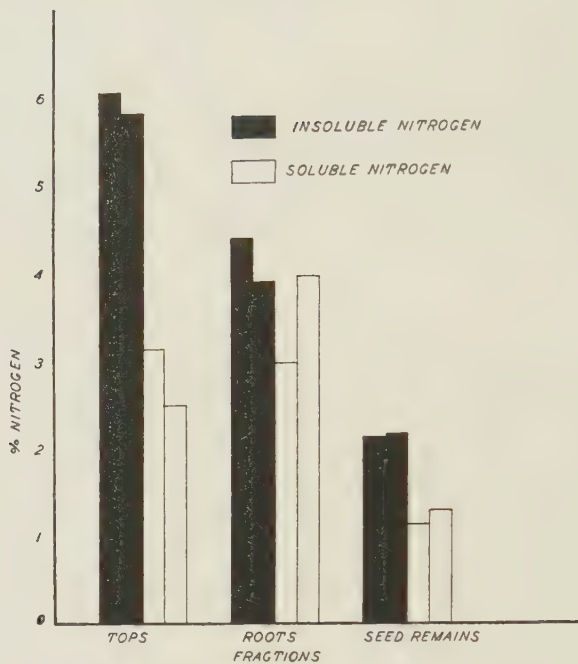


FIG. 7. Nitrogen content of 7-day-old Marquis wheat seedlings. Left bar, with phosphorus; right bar, without phosphorus.

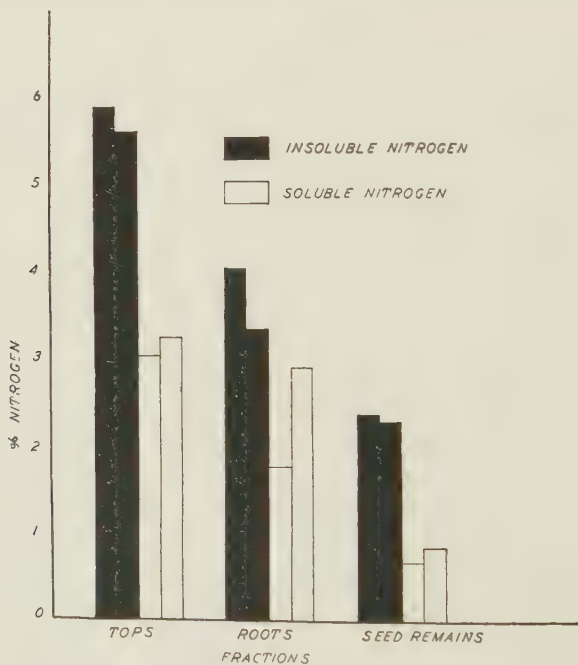


FIG. 8. Nitrogen content of 7-day-old Poso wheat seedlings. Left bar, with phosphorus; right bar, without phosphorus.

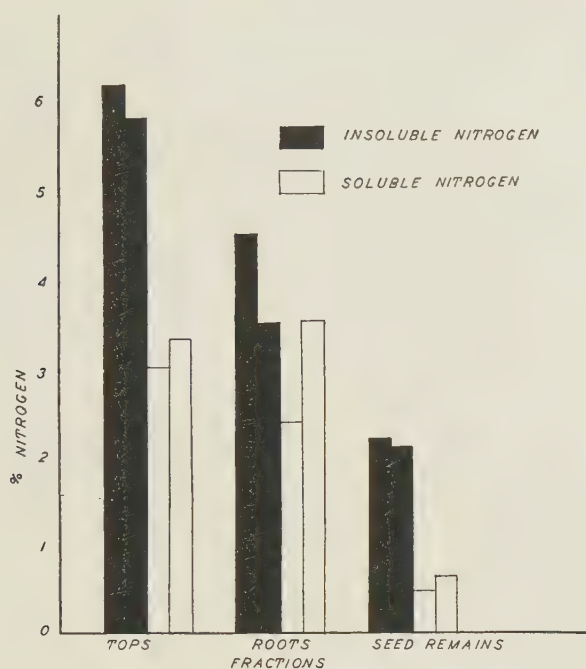


FIG. 9. Nitrogen content of 7-day-old Kubanka wheat seedlings. Left bar, with phosphorus; right bar, without phosphorus.

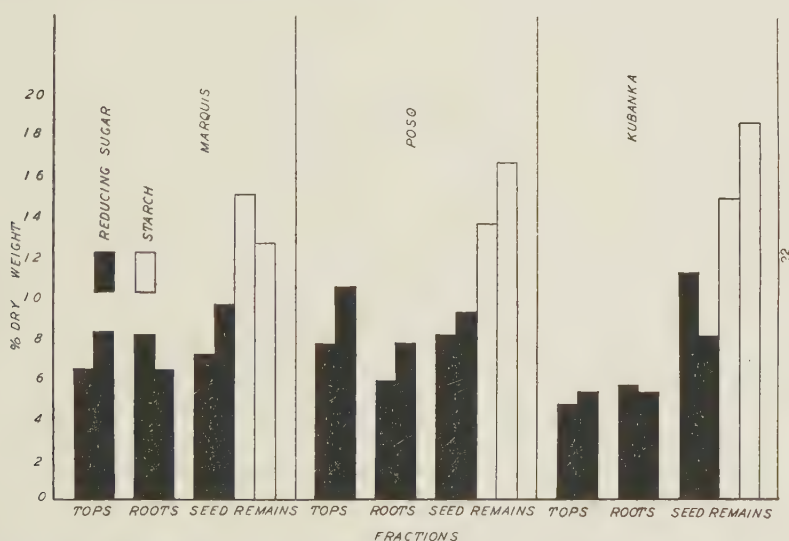


FIG. 10. Carbohydrate content of 7-day-old wheat seedlings. Left bar, with phosphorus; right bar, without phosphorus.

pointed out above, however, form a more or less permanent union with phosphorus. This bound phosphorus is then not free to take part in the respiratory process. In *Elodea canadensis* and in wheat seedlings LYON (12) was able, by the application of phosphate, to obtain an increase in the production of carbon dioxide amounting to 55 per cent. for the former and 35 per cent. for the latter plant. This increase for wheat seedlings was much greater than was obtained in the present work. Here the increase was, in most cases, approximately 6 per cent. This increase bears a very direct relation to certain chemical constituents of the plant which will be discussed later. LYON gives no explanation as to the treatment of the wheat seedlings except to say that phosphate was supplied.

CHEMICAL COMPOSITION

According to ECKERSON (4, 5), anything that interferes with the reductase activity of a plant causes an interruption of the general metabolism of the plant. The plant becomes high in carbohydrates and has the general appearance of a nitrogen-starved plant (9). Although there are plenty of nitrates present, they are not reduced owing to the low reductase content of the plant. Also plants deficient in sulphur (3, 22), calcium (21), or phosphorus (4, 5, 15) have the appearance of nitrogen-starved plants. In the absence of these elements the reductase content is low.

In the 7-day-old wheat seedlings, the plants in the complete nutrient solution as compared with those in the minus-phosphate solution were somewhat taller, had a slightly larger root system, and were somewhat more succulent. Table V shows that the differences between the phosphorus-deficient plants and those grown in a complete nutrient solution are very similar to the differences obtained with the tomato by MACGILLIVRAY (15). In all three varieties of both species studied there was, in most cases, an accumulation of reducing sugars in the tops and roots of the phosphorus-deficient plants. There was no sucrose or starch found in the tops or roots of the plants at any time. There was considerable reducing sugar and starch left in the seed-remains indicating that not all of the reserves were used. These carbohydrates in the seed-remains represent part of the original stored starch that was not moved out. From table V it is apparent that the deficiency of phosphorus did not interfere with the digestion of the starch.

In phosphorus-deficient tomato plants, according to MACGILLIVRAY (15) and ECKERSON (4, 5); in sulphur-deficient tomato plants, according to NIGHTINGALE *et al.* (22); and in sulphur-deficient soy bean plants, according to EATON (3), there is an accumulation of soluble nitrogen and a decrease in insoluble nitrogen. Table V and figures 7, 8, and 9 show that this is also true of phosphorus-deficient wheat seedlings. The percentage of insoluble nitrogen was always greater in the seedlings grown in a complete nutrient

solution, while the soluble nitrogen was greater in the phosphorus-deficient plants. The lack of phosphorus probably did not interfere with the hydrolysis of the proteins stored in the seed, but in the resynthesis of these proteins and in the formation of new proteins from the nitrates of the nutrient solution the phosphorus was apparently limiting. This limiting action was probably of a dual nature: (1) those proteins containing phosphorus could not be synthesized, and (2) the reduction of nitrates was hindered owing to the low reductase content (5) of the plants.

Table V shows that the plants from a complete nutrient solution contain more than twice as much total phosphorus as the plants from a nutrient solution lacking phosphorus. This was expected, but the distribution of the phosphorus in the insoluble and soluble forms offers a still greater point of contrast. Except in the case of the Poso, in the plants grown on a complete nutrient solution about 50 per cent. of the total phosphorus was in the soluble form, while in the plants grown on the phosphorus-deficient nutrient solution only about 5 per cent. or less of the total phosphorus was in the soluble form. The soluble phosphorus determinations were run on the alcoholic extract, and, according to LEVENE (11), all of the phosphatides are extracted by hot alcohol. If this is true then there was only a very small trace of phosphatides in the phosphorus-deficient plants.

RELATION BETWEEN RESPIRATION AND CHEMICAL COMPOSITION

Fundamentally respiration is not a complex reaction. It is an oxidation-reduction reaction wherein one substance is oxidized, and another is reduced with the liberation of energy. In the case of most plants carbon is oxidized and oxygen reduced. The factors conditioning this oxidation-reduction are many and complex, however, and not well understood. It is affected by the formation of a suitable substrate, by the presence of enzymes, and by many and varied environmental conditions. SPOEHR (28) has pointed out that when carbohydrates are present in sufficient amounts, respiration is independent of carbohydrates, but proportional to the amino acids present, which, he claims, act in a catalytic manner. This does not seem to hold in the present case.

From tables IV and V the following relations between respiration and chemical composition can be drawn. Where respiration is most active there is (a) a greater insoluble nitrogen content, (b) a smaller soluble nitrogen content, (c) a smaller reducing sugar content, and (d) a greater phosphorus content, both soluble and insoluble. PALLADIN (23) states that, "When there is sufficient amounts of carbohydrates present the quantity of carbon dioxide exhaled by plants is directly proportional to the quantity of insoluble protein contained in them." In seedlings, the quantity of insoluble proteins is a measure of respiration.

Phosphorus, then, seems to influence respiration in two ways: (1) directly, by its association with carbohydrates to form a suitable substrate for the respiratory enzymes, and (2) indirectly, as it limits the amount of protein synthesis, and thereby the rate of growth.

Summary

1. Marquis spring wheat was grown to maturity in the greenhouse in a phosphorus-deficient nutrient solution. These plants yielded fewer and lighter seeds, which had a slightly higher protein content and a lower starch and phosphorus content than plants grown to maturity in a complete nutrient solution.

2. The respiration behavior of the above seeds was studied during germination on a nutrient solution lacking phosphorus and on a complete nutrient solution. The addition of phosphorus to the nutrient solution caused an increase in the production of carbon dioxide of about 10 per cent., in most cases, regardless of whether the seeds came from plants grown on the phosphorus-deficient nutrient solution or from plants grown on the complete nutrient solution.

3. Two species of wheat, including three varieties, were grown in nutrient solutions with or without phosphorus for a period of 7 days and the respiration measured during this period. At the end of the 7-day period other plants treated the same were analysed for reducing sugars, starch, soluble and insoluble nitrogen, and soluble and insoluble phosphorus. From this study the following conclusions are drawn:

a. The wheat plants grown on a complete nutrient solution respired approximately 6 per cent. more carbon dioxide than those plants grown on a nutrient solution lacking phosphorus.

b. Those plants grown on a complete nutrient solution as compared with those on a nutrient solution lacking phosphorus contained (1) more insoluble and less soluble nitrogen, (2) less reducing sugars, and (3) more insoluble and more soluble phosphorus.

c. No starch was found in the tops or roots of the wheat seedlings in any case. No sucrose was found.

d. In the present study respiration is not limited by the lack of available carbohydrates, but is limited by the lack of phosphorus.

e. Phosphorus, then, seems to influence respiration in two ways: (1) directly, by its association with carbohydrates to form a suitable substrate for the respiratory enzymes, and (2) indirectly, as it limits protein synthesis.

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